UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: William Miller (16)
Registration Division (TS-767)
and
Environmental Fate Branch
Hazard Evaluation Division (TS-769)

SUBJECT: EPA Reg.#1471-RER; Bromethalin; teratology and delayed neurotoxicity studies
CASWELL#561BB Accession#247447

Recommendations:

1. The registration is not toxicologically supported. Toxicology Branch is concerned about the maternal toxicity (abortion) at the lowest level tested, 0.1 mg/kg, in the rabbit teratology study. A NOEL for this effect is required. Additionally, an applicator exposure assessment is required. Consult with EFB regarding the details of the exposure assessment.

2. The submitted studies are acceptable as Core-Minimum Data.

Review:

1. Previously Submitted Toxicity Data

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Study	Material	Results	TOX Category	CORE Grade
Acute oral LD_{50} - mouse	TECH in acacia suspension	LD_{50} = 28.9 mg/kg (female) = 35.9 mg/kg (male)	I	Minimum
Acute oral LD_{50} - mouse	TECH in Peg-200	LD_{50} = 8.1 mg/kg (female) = 5.3 mg/kg (male)	I	Minimum
Acute oral LD_{50} - rat	TECH in acacia susepnsion	LD_{50} = 9.1 mg/kg (female) = 10.7 mg/kg (male)	I	Minimum
Acute oral LD_{50} - rabbit	TECH in Peg-200	LD_{50} = 2.0 mg/kg (female) = 2.4 mg/kg (male)	I	Minimum
Acute oral LD_{50} - cat	TECH in capsule	LD_{50} = 100 mg/kg	I	Minimum
Acute oral LD_{50} - cat	TECH in Peg-200	LD_{50} = 1.8 mg/kg	I	Minimum
Acute oral LD_{50} - dog	TECH in capsule	LD_{50} = > 5 < 10 mg/kg	I	Minimum
	TECH in Peg-200	LD_{50} = 4.8 mg/kg	I	Minimum
Acute oral LD_{50} - monkey	TECH in Peg-200	LD_{50} = 5 mg/kg	I	Supplement
Acute dermal LD_{50} - rabbit	TECH	LD_{50} = > 2 gm/kg	II	Minimum
Acute dermal irritation rabbit	TECH	No irritation	IV	Minimum
Acute dermal LD_{50} - rabbit	TECH	LD_{50} = > 1 gm/kg	II	Minimum

Study	Material	Results	TOX Category	CORE Grade
Acute dermal irritation - rabbit	TECH	No irritation	IV	Minimum
Acute eye irritation - rabbit	TECH	Slight irritation for 72 hrs. Slight corneal dullness cleaned by 24 hrs.	III	Minimum
Acute inhalation LC_{50} - rat	TECH	LC_{50} = 0.024 mg/L (M & F)	I	Minimum
Acute dermal LD_{50} - rabbit	1% formulation in degerminated corn flour	LD_{50} = 4306 mg/kg	III	Minimum
Acute dermal LD_{50} - rat	1% formulation in degerminated corn flour	LD_{50} = > 2.0 gm/kg	III	Minimum
Primary dermal irritation - rabbit	1% formulation in degerminated corn flour	No irritation	IV	Minimum
Primary eye irritation rabbit	1% formulation in degerminated corn flour	Corneal dullness cleaned by 3 days. Irritation cleared by day 7.	III	Minimum
Acute oral LD_{50} - rat	.005% Bait	LD_{50} > 500 mg/kg (HDT)	III	Minimum
Acute dermal LD_{50} - rabbit	.005% Bait	No irritation; no deaths; LD_{50} > 2000 mg/kg	III	Minimum

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Study	Material	Results	TOX Category	CORE Grade
Primary eye irritation - rabbit	.005% Bait	Slight iritis, corneal dullness, mild conj. Normal within 3-7 days.	III	Minimum
Acute inhalation LC_{50} - rat	.005% Bait	No deaths; LC_{50} > .122 mg/L (analytical) or 144.9 mg/L (nominal)	I	Minimum
Teratology - rat	EL-614 Tech.	No fetal or maternal toxicity at 0, 0.05, .1, .2 or .4 mg/kg (pilot study)		Supplementary
Teratology - rat	EL-614 Tech.	Doses of 0.6, 0.8 and 1.0 mg/kg resulted in excessive maternal toxicity.		Supplementary
Teratology - rat	EL-614 Tech.	Negative up to 0.5 mg/kg maternal toxic LEL = .5 mg/kg Fetotoxic LEL = .5 mg/kg Maternal & Fetotoxic NOEL = .3 mg/kg		Minimum
Reversibility of Control Nervous System Lesions from Chronic Bromethalin Admin.	EL-614 Tech.	Obvious signs of toxicity at .23 mg/kg/day can be reversed.		Supplementary
Mutagenic ames	EL-614 Tech.	Positive mutagenic results in 4 strains of S. typhimurium.		Acceptable
Mutagenic, DNA repair	EL-614 Tech.	Negative without activation and 5 strains with activation.		Acceptable
Mutagenic, mouse lymphoma cell forward mutation assay	EL-614 Tech.	No induction of DNA synthesis. Not mutagenic in mouse lymphoma cells.		Acceptable
Mutagenic, sister chromatid exchange in bone marrow of chinese hamsters	EL-614 Tech.	Not mutagenic.		Acceptable

2. New Toxicity Data Submitted with this Registration.

a. The toxicity of bromethalin (EL-614) to hen chickens in a 14-day acute oral study (Elanco #A00881; 5/5/81)

Test Material: Bromethalin (EL-614); Lot#G40-T77-037; 95.4% purity

Groups of 10 female leghorn chickens, 19 weeks old, received by oral gavage (5.0 ml/kg) doses of 0, 8.0, 11.0, 16.0, 22.5 or 30.0 mg/kg of test material. Observation was for 14 days.

Results: LD_{50} = 8.3 mg/kg (5.2 - 13.1 mg/kg)

Toxic Signs: Ataxia, loose feces, prostation

Body Weight: Survivors regained lost body weight in groups $\leq$ 16.0 mg/kg

Food Consumption: Food consumption was comparable to changes in body weight.

Necropsy: Not performed.

Classification: Core-Minimum Data

b. The toxicity of bromethalin (EL-614) to hen chickens in a 24-day acute oral delayed neurotoxicity study (Elanco #A00981; 3/32)

Test Material: Bromethalin (EL-614); Lot#B31-72C-18R (96.3% purity); G40-T77-037 (95.4% purity)

White rock strain hen chickens, 44 weeks old, were used in the study. The vehicle control group (10 animals) received PEG-400, 5.0 ml/kg. The bromethalin treated group (30 animals) were initially dosed with 9.0 mg/kg and redosed on day 3 with 15.0 mg/kg, 5.0 ml/kg. The positive control group (10 animals) were dosed with 431.0 mg/kg, 0.5 ml/kg of TOCP. Observation was for 24 days.

Results:

No deaths occurred in the vehicle control group or positive (TOCP) control group. In the bromethalin treated group, two birds died and two moribund bird were killed on day 5. Two moribund birds were killed on day 7 and two moribund bird were killed on day 14.

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No toxic signs were observed in the vehicle control group. Ataxia was observed in the bromethalin treated birds from days 1-19 and in the TOCP group from days 12-24. Emaciation was noted in some birds of the bromethalin treated group and the TOCP group (Table 1).

TABLE 1. SUMMARY OF SIGNS OF TOXICITY AND MORTALITY OBSERVED IN HEN CHICKENS (*Gallus domesticus*) THAT RECEIVED TWO ACUTE ORAL DOSES OF BROMETHALIN OR TRI-O-CRESYL PHOSPHATE (TOCP). STUDY A00981.

Dose[1]	Test-Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Vehicle Control (PEG-200)[2]																								
N = 10																								
Normal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bromethalin (9.0 mg/kg)[2]																								
N = 10																								
Normal	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Ataxic	±(1)	±(5)	±(6)	±(11)	±(17)	±(12)	±(10,	±	±(9)	±(6)	±(5)	±(4)	±(3)	±(1)	±	±	±	±	±					
Emaciated													±(3)	±(3)	±(1)	±	±	±	±	±	±	±	±	±
No. of Deaths					4		2							2										
Positive Control (431.0 mg/kg of TOCP)																								
N = 10																								
Normal	+	+	+	+	+	+	+	+	+	+	+	±	±	±										
Ataxic												±(3)	±(7)	±(10)	+	+	+	+	+	+		+	+	+
Emaciated														±(1)	±	±	±	±	±	±	±(2)	±		±(3)

+ = All birds responded.
± = One or more birds responded; number in parentheses is the number of animals responding. Lack of a number in parentheses means number of respondents is unchanged.
Absence of Notation = No birds responded.

[1]The dose volume for the control group and bromethalin treatment group was 5.0 ml/kg. Dose volume for the positive control group was 5 ml/kg
[2]All birds were redosed at the end of test-day 3. The bromethalin treatment group was redosed with 15.0 mg/kg, and the vehicle control group was redosed with PEG-200.

Mean body weight values of the bromethalin treated group were significantly reduced from day 7-21 in comparison to the controls. Mean body weight values of the TOCP group were significantly reduced during the entire 24 day period in comparison to the controls.

Food consumption values of the bromethalin treated group were decreased from 4-7 days, and began to increase (although still lower than the controls) from day 8-24 in comparison to the controls. The food consumption values of the TOCP birds were decreased in comparison to the controls for the entire study.

The neurological lesion characterized by bromethalin was spongy degeneration in the brain and spinal cord. TOCP produced axonal degeneration in the spinal cord and sciatic nerve. The bromethalin lesion appeared to be a vacuolation of the myelin sheaths surrounding the axons.

The TOCP lesions directly damaged the axons producing axonal breakdown and swelling.

Conclusion:

Bromethalin did not produce a TOCP type acute delayed neurotoxicity.

Classification: Core-Minimum Data

c. A pilot teratology study with bromethalin (EL-614) in the Dutch Belted Rabbit (Elanco #B7131; March, 1982)

Test Material: Bromethalin; Lot#B31-72C-18R

Groups of 5 pregnant Dutch Belted rabbits received by gavage oral doses of 0, 0.25, 0.5, 1.0 and 2.0 mg/kg of test material on days 6 through 18 of gestation. The dams were sacrificed on gestation day 28. Parameters evaluated included toxic signs, mortality, necropsy results, food consumption, body weight, corpora lutea, implantations, resorptions, number of fetuses, fetal viability, and external anomalies.

Results:

One, 4 and 2 rabbits died in the 0.5, 1.0 and 2.0 mg/ kg groups, respectively.

At necropsy, the female of the 0.5 mg/kg group had pneumonia; two females of the 1.0 mg/kg group had gastric trichobezoars (hair balls); one female of the 1.0 mg/kg group had acute upper respiratory tract infection; one female of the 1.0 mg/kg group had pitting of the kidneys; one female of the 2.0 mg/kg group had pitting of the kidneys and one female in this group had no gross lesions. Necropsy at termination showed purulent material in the uterus of one control female and congested lungs in one female of the 2.0 mg/kg group.

No toxic signs were observed in dams of the control and 0.25 groups. In the 0.5, 1.0, and 2.0 mg/kg groups, the rabbits displayed weakness, decreased muscle tone, nasal discharge, labored respiration and prostation.

Food consumption and body weight was decreased in dams that died.

There were no effects on litter size, implantations, and resorptions.

There were no external abnormalities. Small fetuses were noted in one female of the 0.5 mg/kg group.

Conclusion:

It appears from the data that 0.5 mg/kg of bromethalin should be the high-dose in the full teratology study.

Classification: Supplementary Data

d. A teratology study with bromethalin (EL-614) in the Dutch Belted Rabbit (Elanco #B7141; March , 1982)

Test Material: Bromethalin; Lot#B31-72C-18R

Groups of 15 pregnant Dutch Belted rabbits were orally gavaged with 0, 0.1, 0.25 and 0.5 mg/kg bromethalin on gestation days 6 through 18. The dams were sacrificed on gestation day 28. Parameters evaluated included toxic signs, mortality, necropsy findings, food consumption, body weight, corpora lutea,

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implantations, resorptions, number of fetuses, fetal viability, fetal weight, fetal sex ratio, and external, visceral, and skeletal abnormalities.

Results:

Two rabbits in the 0.5 mg/kg group died. Necorpsy revealed that one dam had pneumonia and the second dam had an acute upper respiratory tract infection. There were four abortions; two at 0.5 mg/kg, one at 0.25 mg/kg and one at 0.1 mg/kg. Toxic signs in the 0.25 and 0.5 mg/kg groups included nasal discharge, loss of muscle tone, weakness, decreased respiration, coolness, and prostation. Food consumption and body weight were unaffected by treatment. Twelve, 12, 11, and 12 dams of the 0, 0.1, 0.25 and 0.5 mg/kg groups, respectively, were pregnant. Surviving females available for teratology evaluation were 12, 11, 10 and 8. Live litter size, corpora lutea, resorptions, fetal weight, and fetal sex distribution were not affected by treatment.

There were no external abnormalities. One control fetus had internal hydrocephalus. The same skeletal variations occurred with similar frequency in all groups.

Conclusion:

Bromethalin was not teratogenic in rabbits at dosages up to 0.5 mg/kg. The fetotoxic NOEL is 0.5 mg/kg. A maternal toxic NOEL was not established.

Classification: Core-Minimum Data

W/RD for LDC
William Dykstra 8/16/82

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